PHASE VARIATION IN *SALMONELLA TYPHIMURIUM* SW629.

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by
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Sal. typhimurium SW629 is the same strain with what has been reported by Seligmann et al. (1945) and by Edwards et al. (1954) as a strain which has poorly motile and faintly anti-i agglutinable phase 1, called X-phase, and normal 1.2-phase 2. The present report deals with the similarity, genetypical as well as phenotypical, of the mode of phase variation in this strain with that in SW1061 (Iino, 1956-i).

MATERIALS AND METHODS.

The culture of SW629, used in this experiment, has been maintained in a nutrient agar stab at room temperature.

For the comparative study, *Sal. typhimurium* strain TM2 (i:1.2), typical diphasics, and SW1061, a monophasic mutant of TM2 with non-motile phase 1 and 1.2-phase 2, were used. In transductional analysis, *Sal. heidelberg* SW1092 (r:1.2), which has Fla$^-$ linked to H_1, was used as a recipient.

Methods of antigen type test and transduction were the same as described in the previous report (Iino, 1956-i). Leifson's method (Leifson, 1951) was emploied for flagellar staining.

EXPERIMENTAL RESULTS.

Antigen reactions of SW629. H-antigen reactions of SW629 were examined followed to the description by Edwards et al. (1954). When the cultures were streaked on EMB-galactose plates, some colonies grown agglutinated with anti-1.2 serum but not with anti-i serum, and the others agglutinated neither anti-i nor anti-1.2 serum (X-colonies of Seligmann et al. 1945).

Microscopical observation of hanging drpps and flagellar stains showed that the cells of anti-1.2 agglutinable colony are mostly motile and flagellated, whereas X-phase cells are mostly non-motile and non-flagellated. When two types of colonies were respreaded on petri dishes with MGA media, anti-1.2 agglutinable colonies formed swarms with few compact colonies, while X-phase colonies formed compact colonies mixed with few swarms. Many compact colonies produced sectorial swarms in prolonged cultures (2 to 3 days at 37C).

A compact colony (X-phase colony) was isolated to a nutrient agar slants. after one or two day culture at 30C, antigen types were examined by slide agglutination test. As controls, parallel cultures of TM2 originated from i-phase colony and SW1061 from non-motile colony were used. The TM2 culture agglutinated strongly with anti-i serum, but both SW629 and SW1061 didn't. Tube dilution test gave the same result; among dilution series from 1/10 to $1/10 \times 2^{12}$ of original anti-i serum, i-phase of TM2 agglutinated with 1/10 to $1/10 \times 2^{10}$ antiserums, but SW629 and SW1061 did not show H-agglutination in any concentrations tested.

The slant cultures were transferred to penassay broth, streaked on EMB-galactose plates, plated on plain or anti-1.2 MGA media, or spotted on anti-1.2 MGA deep tubes. The rest of the cultures were suspended in distiled water and used for flagellar staining. Microscopical observation of the stained bacteria showed that the slant culture of SW629 was the mixture of flagellated and non-flagellated cells. The frequency of non-flagellated cells in a sample was 0.58 (122 among 210). Antigen type of 20 colonies grown on the EMB-galactose plate was as follows; 8 among 20 agglutinated with anti-1.2 serum but not with anti-i serum, and other 12 colonies agglutinated neither with anti-1.2 serum nor with anti-i serum. On the MGA plates, 175 compact colonies and 123 swarms were recovered (the frequency of compact colonies was 0.59, which coincides well with the frequency of non-flagellated cells in the culture), whereas in the anti-1.2 MGA plates all of 312 colonies grown were compact; that is,

the swarms in the plain MGA plate were 1.2 type. The anti-1.2 deep tube cultures of SW629, however, produced swarm which agglutinated distinctly with anti-i serum. The culture from the swarm has maintained i:1.2 diphasic character in serial transfer through penassay broth.

The observations on slant culture were repeated on three other single colony isolations of the X-phase. They all gave the same result with the culture described above, except that the proportion of phase 2 cells in cultures differed in different slants.

Selection of i-phase by anti-1.2 MGA deep tubes was repeated with larger number of X-phase cultures. Each of 25 single colonies of X-phase and the same number of single colonies of non-motile phase of SW1061 was cultured on a anti-1.2 MGA deep tube. 23 among 25 tubes of SW629 produced swarms which agglutinate with anti-i serum, on the contrary only 6 among 25 produced swarms in SW1061 cultures.

From these results, it is summarized that SW629 is the mixture of Fla$^-$ cells and Fla$^+$(1.2) cells, which interchange each other. The interchange is parallel with phase variation in diphasic strain and Fla$^+$(1.2) $\rightleftarrows$ Fla$^-$ interchange in SW1061. The culture also contains few Fla$^+$(i) cells which have been produced by reversion of Fla$^-$ cells. The frequency of Fla$^+$(i) production in SW629 is higher than in SW1061, but not high enough as can be recovered without mass selection by anti-1.2 MGA media.

Transductional analyses. A factor which inactivates flagellar production in phase 1 was analyzed by the same method used in transductional analysis of the monophasics of SW1091; that is, SW629 was used as donor and SW1092 as recipient, and Fla_{1092} transductions were screened from brushes on MGA plates. The results were summarized in Table 1. 3 i:1.2 types and 32 (r):1.2 types were obtained among 332 Fla_{1092} transductions, besides r:1.2

and ($\bar{i}$):1.2 types (($\bar{x}$):1.2 indicates a type which has Fla$^-$ phase 1 and 1.2 phase 2 ~~which~~ and reverts to produce ~~a~~ phase 1). This result is explained by ~~the~~ an assumption that tha fail of flagellar production in phase 1 is caused not by the genetic change of H_1 itself but by the suppression of H_1 function by a factor which linkes to both H_1 and Fla_{1092}. The factor will be designated as Fla_{h1-2}^- tentatively (and the suppressor in SW1061 as Fla_{h1-1}^-).

In order to test whether Fla_{h1-1} and Fla_{h1-2} are allelic or not, transductions were made between SW1061 and SW629 to both directions. The lysates obtained from SW629(-), SW1061(-) and TM2(i) were diluted to the same titer, $2x10^9$/ml. 0.25 ml of each lysate was mixed with 0.25 ml of penassay broth culture of SW626(-) or SW1061(-) (density of the cells was 10^9/ml), and brushed on anti-1.2 MGA plates. The number of swarms recovered was listed in Table 2. Antigen type of the swarms, sampled 20 clones from each combinations, were tested after isolating to EMB-plates. They were all i. In combinations in which SW629 is the recipient, 68 swarms were recovered when SW629 ~~is~~ was the donor. These swarms may be appeared not by transduction but by reversion. The number of swarms increased when SW1061 ~~is~~ was used as donor, and became highest when TM2(i) ~~is~~ was donor. When SW1061 ~~is~~ was the recipient, none of the swarms were recovered from self-transduction, whereas 28 swarms appeared in SW629 -x, and more than hundred in TM2-x. These results indicate that Fla_{h1-1} and Fla_{h1-2} are not allelic but linked closely. The production of trails were observed only when TM2 is donor. The significance of this phenomenon will be discussed later.

Stability of Fla_{h1-1}^- and Fla_{h1-2}^-. As the result of i-phase screening by anti-1.2 MGA stabs has indicated, SW629(-) revert more frequently to i:1.2 type than SW1061. The difference of the frequency was also observed between -:1.2 clones obtained from SW629 -x SW1092 and SW1061 -x SW1092.

In SW628 -x SW1092, 30 among 32 i:1.2 transductional clones produced phase 1 swarms in the first selection, and the other two produced in the second selection; whereas -:1.2 from SW1061 produced only two reversions among five repeated selections of five -:1.2 clones. Consequently, the difference in the frequency of reversion between SW629 and SW1061 are attributed to the different reversibility of Fla^{-}_{h1-1} and Fla^{-}_{h1-2} .

DISCUSSION.

Phase variation of Sal. typhimurium SW629 is performed between Fla^{-} - phase 1 and 1.2-phase 2. The strain also produces i:1.2 diphasic type by reversion. These behaviors coincide well with the description by Seligmann et. al. (1945) and Edwards et al. (1954) except one point that the difinite i reaction could not be observed on nutrient agar slant cultures in this experiment. The discrepancy on the reaction of agar slant culture may be explained by either one of following two reasons. The first is the difference of media used; the composition of the nutrient agar slant used in this experiment may differ from what has been used by Edwards et al., and could not support flagellar production in phase 1 of SW629. The second explanation is based on the high frequency of reversion of SW629; SW629 reverts frequently to i:1.2 diphasic type, and mass cultures usually contain some proportion of i type cells. The culture reported by Edwards et al. may be the culture which has contained high proportion of i type cells.

Transductional analysis showed that SW629 has potentially active H_1^i but its function is suppressed by a factor, Fla^{-}_{h1-2}. The mode of suppression by Fla^{-}_{h1-2} is quite similar with that of Fla^{-}_{h1-1} in SW1061. In both cases, suppression is on H_1 function but not on H_2. In both cases, the factor linkes to H_1 and Fla_{1092}. The factors, however, are not allelic and also differ in

their frequency of reversion.

The transductions SW629 $\rightarrow$ SW1061 on anti -1.2 MGA media are the first examples in which swarms were obtained but trails were not, from transductions between Fla$^-$ strains. The explanation of the mechanism of trail phenomena in Fla-transductions still allows several alternatives (Lederberg, 1956). However, it is the least common assumption that the first step of the trail production is the expression of the phenotype (flagellar production) before perfect organization of Fla factor into the chromosome of the recipient takes place. Based on this assumption, together with the fact that Fla_{h1-1} and Fla_{h1-2} link closly each other, the failure of the trail production is most plausibly explained that Fla_{h1-1} or Fla_{h1-2} can not express their function unless they are arranged in a chromosome together; that is, they have cis-trans position effect each other.

SUMMARY.

1). Sal. typhimurium SW629 changes its phase between Fla$^-$ (phase 1) and 1.2 (phase 2) in the media tested.

2). The factor (Fla^-_{h1-2}) which supresses the production of flagella in phase 1 links to H_1 and Fla_{1092}, but not identical with the H_1 suppressor (Fla^-_{h1-1}) in SW1061. Fla_{h1-1} and Fla_{h1-2} also link closly each other, and may belong to a cistron, but have different frequency of reversibility.

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Table 1.

Transduction, SW629 (-:1.2) -x SW1092 (Fla^-, R^r:1.2)

Fla_{1092} were used as selective marker.

Experimental no.		1	2	3	4	5	6	Total
Antigen type of transductions	(r):1.2	35	2	33	50	12	13	145
	r :(1.2)	29	40	13	3	49	27	161
	(i):1.2	0	0	1	0	0	1	2
	i :(1.2)	0	0	0	0	1	0	1
	(R):1.2	0	0	1	0	0	1	2
	(I):1.2	11	1	7	9	0	2	30
	Total	75	43	55	62	62	44	341

Table 2.

Number of swarms and trails produced from transductions among TM2, SW629 and SW1061. Swarms were screened on anti-1.2 MGA plates.

Donor	TM2	SW629	SW1061	TM2	SW629	SW1061
Recipient	SW629	SW629	SW629	SW1061	SW1061	SW1061
no. of swarm	218	68	182	114	28	0
no. of trail	over 300	0	0	over 500	0	0